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Metabolic changes in single human muscle fibres during brief maximal exercise

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Changes in high-energy phosphate levels in single human skeletal muscle fibres after 10 s of maximal (all-out) dynamic exercise were investigated. Muscle biopsies from vastus lateralis of two volunteers were collected at rest and immediately post exercise. Single muscle fibres were dissected from dry muscle and were assigned into one of four groups according to their myosin heavy chain (MyHC) isoform content: that is type I, IIA, IIAx and IIXa (the latter two groups containing either less or more than 50% IIX MyHC). Fragments of characterised fibres were analysed by HPLC for ATP, inosine-monophosphate (IMP), phosphocreatine (PCr) and creatine levels. After 10 s of exercise, PCr content ([PCr]) declined by approximately 46, 53, 62 and 59% in type I, IIA, IIAx and IIXa fibres, respectively ($P < 0.01$ from rest). [ATP] declined only in type II fibres, especially in IIAx and IIXa fibres in which [IMP] reached mean values of 16 ± 1 and 18 ± 4 mmol (kg dry mass)$^{-1}$, respectively. While [PCr] was reduced in all fibre types during the brief maximal dynamic exercise, it was apparent that type II fibres expressing the IIX myosin heavy chain isoform were under a greatest metabolic stress as indicated by the reductions in [ATP]. *Experimental Physiology* (2001) **86.3**, 411–415.

Human skeletal muscle fibres have been reported to have fibre type-dependent differences in high-energy phosphate (HEP) content at rest. Most often, type I fibres have been found to contain lower absolute amounts of phosphocreatine (PCr) and ATP compared with type II fibres (Söderlund *et al.* 1992; Greenhaff *et al.* 1994; Sant'Ana Pereira *et al.* 1996). Among pools of type II fibres an increasing resting PCr content has been reported as expression of myosin heavy chain (MyHC) IIX increased (Sant'Ana Pereira *et al.* 1996). Twenty-five seconds of maximal dynamic exercise resulted in almost total depletion of PCr in all fibre types and greater reductions in ATP content in type II fibre groups compared with type I fibres (Sant'Ana Pereira *et al.* 1996; Karatzaferi *et al.* 2001). It was concluded that changes in response to high intensity exercise varied according to the MyHC isoform content of the fibres (Sant'Ana Pereira *et al.* 1996). The observed low post-exercise ATP levels were accompanied by high IMP levels, which were higher for type II fibres, especially the those expressing large amounts of the IIX MyHC isoform (Sant'Ana Pereira *et al.* 1996).

From the evidence of studies of stimulated amphibian fast muscle fibres (Nagesser *et al.* 1992), it could be suggested that accumulation of inosine-5′-monophosphate (IMP), occurs only after PCr levels are depleted. A previous study in human muscle from our own laboratory also confirmed that IMP accumulation had occurred in muscle fibres that had been depleted of PCr (Sant'Ana Pereira *et al.* 1996). However, since after the 25 s dynamic exercise used in that study all fibre populations were depleted of PCr it was not possible to determine those fibres which were subjected to the greatest metabolic stress or the time course of events leading to IMP accumulation.

We hypothesised that in human mixed muscle different fibre populations will be metabolically challenged with a different time course related to their MyHC expression. Therefore we investigated the changes in [HEP] consequent upon a short 10 s period of maximal exercise which we expected to selectively challenge the fast fibre populations.

A preliminary report of some of this work has appeared (Karatzaferi *et al.* 2000).

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Table 1. Metabolite levels (expressed in mmol (kg DM)$^{-1}$) for the different fibre groups before and after 10 s of maximal isokinetic cycling

Rest	*n*	Cr	PCr	IMP	ATP	Post-exercise	*n*	Cr	PCr	IMP	ATP
I	12	30.1 ± 3.6	69.5 ± 15.9	n.d.	23.7 ± 4.8	I	22	66.6 ± 14.3	37.5 ± 7.7	n.d. *	24.7 ± 4.7
IIA	18	37.7 ± 5.3	73.6 ± 10.9	n.d.	25.3 ± 3.2	IIA	11	78.2 ± 6.9	34.3 ± 7.2	10.4 ± 6.0	15.1 ± 5.7
IIAx	8	38.7 ± 4.9	73.3 ± 13.7	n.d.	24.7 ± 2.9	IIAx	12	78.6 ± 4.2	27.4 ± 4.2	16.1 ± 1.3	8.1 ± 2.2
IIXa	8	36.4 ± 6.3	69.6 ± 6.3	n.d.	25.0 ± 4.2	IIXa	7	94.9 ± 13.3	28.3 ± 2.8	17.7 ± 4.0	7.3 ± 2.7

All values are expressed as means ± S.D.; n.d., not detected; * only detectable in two fibres < 0.5 mmol (kg DM)$^{-1}$. Apart from [ATP] in type I fibres, all post-exercise results were significantly different from rest ($P < 0.01$). Refer to text for between-fibre-group comparisons.

METHODS

Subjects

Two generally active healthy volunteers (one male (A); one female (B)), with no history of muscle or metabolic disorders, participated in this study which conformed to the guidelines of the Declaration of Helsinki and had the approval of the ethical committee of Vrije University, Amsterdam, The Netherlands. Their age, body mass and height were 32 and 25 years, 69 and 62 kg, 176 and 174 cm, respectively. From previous analysis it was estimated that subject A had a 48% and subject B had a 56% type I fibre composition. The subjects expressed understanding of the purpose of the study, any known risks, and their right to terminate participation at will, by signing a statement of informed consent.

Experimental protocol

Subjects were familiarised with the exercise protocol on different occasions. On the experimental day, subjects performed 10 s maximal effort at 120 crank revolutions per minute on an isokinetic cycle ergometer. During the course of the exercise, forces exerted on the pedals were continuously monitored by strain gauges mounted in the foot pedals (Beelen & Sargeant, 1991). Muscle samples from vastus lateralis were collected at rest and immediately post exercise.

Power output calculations

Power data were calculated as described by Beelen & Sargeant (1991). Briefly, the power output for each leg per revolution was calculated by multiplying the effective (tangential) force and instant velocity. The average between left and right peak power values represented the peak power for each revolution.

Muscle sample collection and analyses on single fibres

Muscle samples from vastus lateralis were collected, under local anaesthesia, using a Bergstrom-type biopsy needle (UCH, diameter 5.5 mm) with suction. The resting biopsy was collected from the right leg with the subject lying on a couch and the post-exercise biopsy was collected from the left leg with the subject still sitting on the bike. For the biopsies in both legs, small incisions in the skin and fascia were made under local anaesthesia and were temporarily covered with aseptic gauze. Biopsies were collected at a depth of 2–3 cm. Samples were immediately frozen in liquid nitrogen and freeze dried overnight. Freeze-dried muscle was stored desiccated at −80 °C. Single muscle fibres were dissected under conditions of controlled ambient temperature and relative humidity (21 °C and 25% relative humidity) and handled as previous described for subsequent analysis (Karatzaferi *et al.* 1999). In brief, serial cryostat sections (10 μm thick) of gelatine-embedded single fibre fragments were characterised by fixed-acid labile myofibrillar ATPase (mATPase) activity after pre-incubation at pH 4.4 and 4.6 (adapted from Brooke & Kaiser, 1970). Optical density (OD) readings from histochemically treated fibre sections were collected using an image analysis system. The basic procedure has been described elsewhere (Sant'Ana Pereira *et al.* 1995). All OD measurements were performed within less than 48 h from the time of histochemistry. Fibres were classified into four groups, type I fibres, type IIA fibres and two groups for hybrid type IIAX fibres, namely IIAx and IIXa, estimated to comprise 15–50% and 50–100% type IIX MyHC isoform, respectively.

Analysis of metabolite levels

Fragments of characterised single fibres were analysed for levels of ATP, IMP, PCr and creatine (Cr) by using reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) photometric detection, following overnight extraction in 60% methanol (Karatzaferi *et al.* 1999). Metabolite concentration (noted in square brackets), [ATP], [IMP], [PCr] and [Cr] values, are expressed in mmol (kg dry mass (DM))$^{-1}$. The values were adjusted for total creatine (TCr) content determined from type I and type II fibre pools, for each subject individually. Muscle TCr content is frequently used as a reference base for reporting intracellular metabolite content (Sabina *et al.* 1983; Harris *et al.*1976; Stathis *et al.*1994; Sahlin *et al.*1997). This allows for compensation of possible weighing errors or errors due to any differences in metabolites along the fibre's length or due to any variability in biopsy composition arising from exercise hyperaemia (Harris *et al.*1976; Rehunen *et al.* 1982).

Statistics

Values were presented as means ± standard deviation (S.D.). Non-parametric statistics were employed and differences in metabolites per fibre group and time were assessed by Kruskal-Wallis non-parametric analysis of variance. *Post hoc* comparisons were performed by a Mann-Whitney *U* test for independent samples as suggested by Sheskin (1997). Relationships between some compounds were investigated using Pearson's correlation analysis (*r*). All statistical analyses were performed at a $P < 0.05$ significance level, using a commercially available statistical package (SPSS 6.0 for Windows, SPSS Inc. 1993).

RESULTS

Power output

Power data were calculated per revolution, providing us with peak power per revolution. The two subjects were able

to produce their maximal peak power levels within 2–3 revolutions from the start of the 10 s exercise bout: 1370 and 1100 W for subjects A and B, respectively. By the end of the 10 s maximal cycling period their peak power had declined by 20% and 26%, respectively.

High-energy phosphates

Rest. No significant differences were found between fibre groups in resting [ATP] and [PCr] (Table 1). Type I fibres had a lower [Cr] than type IIA, IIAx and IIXa fibres ($P < 0.05$). IMP was not detectable in resting samples for all fibre types (Table 1).

Post-exercise. Post-exercise [PCr] in type I was not different from type IIA fibres (Table 1). However, the △ PCr was larger in type IIA compared to type I fibres ($P < 0.05$). In type I fibres post-exercise [PCr] was higher than in IIAx and IIXa fibres ($P < 0.01$, Table 1). Post-exercise [PCr] in type IIA fibres was higher than in IIAx and IIXa fibres ($P < 0.05$, Table 1). There was no difference in post-exercise [PCr] between the IIAx and IIXa fibres (Table 1).

Post-exercise [ATP] was significantly higher in type I fibres than in all type II fibres ($P < 0.01$, Table 1 and Fig. 1). [ATP] in type IIA fibres was significantly higher ($P < 0.01$) than in the IIAx and IIXa fibres. Post-exercise [IMP] was not detectable in most type I fibres (only detectable in 2 fibres < 0.5 mmol (kg DM)$^{-1}$) (Table 1, Fig. 1). Among type II fibres, the IIA had a lower [IMP] than the IIAx and IIXa fibres ($P < 0.01$, Table 1, Fig. 1).

As expected, post-exercise [ATP] and [IMP] were negatively related in all fibres ($r = -0.83$, $P < 0.01$). A strong positive relation was observed between post-exercise [PCr] and [ATP] within type II fibres ($r = 0.63$, $n = 30$, $P < 0.01$). Moreover, [PCr] was negatively related to [IMP] within type II fibres ($r = -0.79$, $n = 30$, $P < 0.01$, Fig. 2). In no case, however, was PCr totally depleted. The lowest values observed were > 20 mmol (kg DM)$^{-1}$.

DISCUSSION

The present data showed that following 10 s of maximal dynamic exercise all characterised muscle fibre populations exhibited a reduction in [PCr]. In type I fibres the reduction was to 54% of resting values. There was a greater reduction in type IIA fibres to 47% and a further reduction to ~39% of resting values in the IIX expressing fibre populations. The systematic decrease in PCr from I to IIA to IIAx and IIXa was associated with coherent changes in [ATP] and [IMP]. In type I fibres which had the smallest decline in [PCr] there was no significant change in [ATP] and no detectable [IMP] production. In the IIA, IIAx and IIXa fibre populations [ATP] systematically decreased by ~40, 68 and 71%, respectively. The orderly decrease in [ATP] was reflected in a concomitant increase in [IMP] which represented 0, 41, 67 and 71% of the sum of ATP + IMP (which mainly determines the total nucleotide pool) in the type I, IIA, IIAx and IIXa fibre populations, respectively, after 10 s of maximal exercise (Fig. 1).

It has been previously argued that [ATP] is closely related to loss of power in metabolically induced fatigue of mammalian skeletal muscle (de Haan & Koudijs, 1994). It is interesting to note that in the present study a 23% reduction in peak power output was associated with marked reductions in [ATP] in the IIX-expressing fibres, but with lesser reduction in type IIA fibres and with none at all in type I fibres. Taken together with data from a previous study of 25 s maximal exercise (Karatzaferi *et al.* 2001), it can be seen that following a further 15 s of exercise there was little further change in [ATP] in the IIX-expressing fibres while the data for the IIA fibres suggested a continuing reduction in [ATP] (Fig. 3). In agreement with this, Casey *et al.* (1996), employing 30 s of maximal isokinetic cycling, observed a greater ATP utilisation in type II fibres in parallel with a greater PCr utilisation, compared to type I fibres (47% decline in ATP and 84% decline in PCr levels in type II fibre pools). The

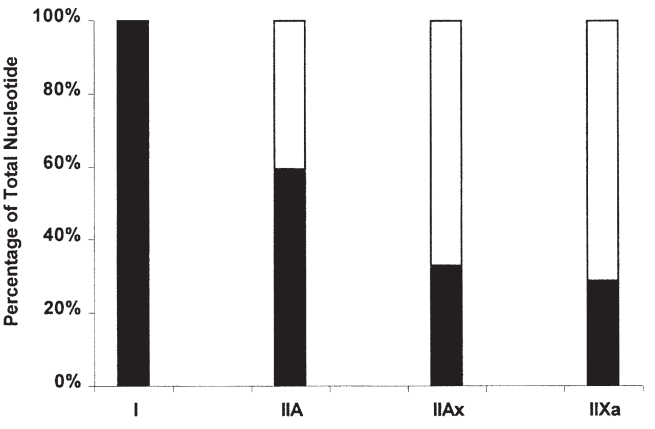

Figure 1

Mean post-exercise [ATP] and [IMP] expressed as a percentage of the sum of nucleotides (filled and open portions of the bars, respectively). S.D. values were omitted for clarity but they were (±) 0.3, 22, 6 and 7% for type I, IIA, IIAx and IIXa fibres, respectively.

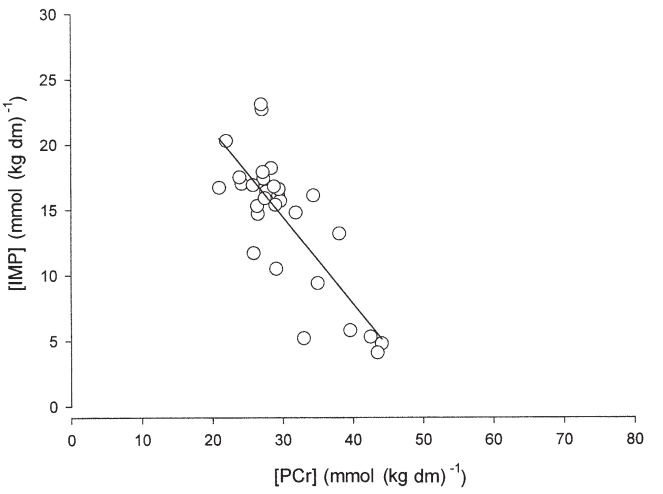

Figure 2

Post-exercise [IMP] plotted against [PCr] for type II fibres ($n = 30$). The correlation coefficient was $r = -0.79$ ($P < 0.01$).

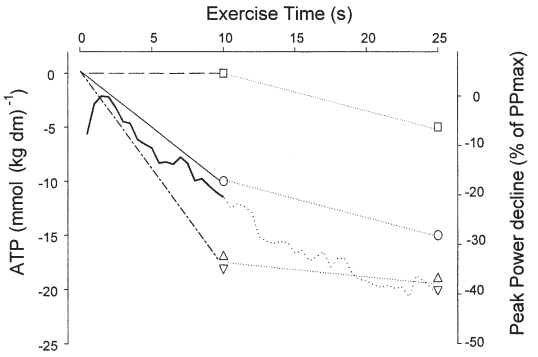

Figure 3

Mean decline in [ATP] for type I (□), IIA (○), IIAx (△) and IIXa (▽) fibres for the present study (10 s exercise) and a previous study (25 s exercise, Karatzaferi *et al.* 2001). The metabolic changes were combined with the power profile of one subject (A) who participated in the present study (continuous line). The same subject performed 25 s of exercise on another occasion (dotted line).

absence of any change from resting levels of ATP in type I fibres after 10 s of exercise suggested that there may have been no fatigue-inducing challenge in those fibres. After 25 s of exercise, however, ATP was significantly reduced, even in this type I fatigue-resistant fibre population, with a concomitant production of IMP (Fig. 3).

It has been suggested that IMP and ammonia formation serve to maintain energy balance by keeping [AMP] low so ATP formation through the myokinase reaction is favoured. IMP may also activate phosphorylase b while the formation of ammonia may stimulate glycolysis by phosphofructokinase activation and buffer the H^+ increases (Lowenstein, 1972; Sahlin *et al.* 1978; Meyer & Terjung, 1979; de Haan & Koudijs, 1994). In humans after high intensity knee extensions (Jansson *et al.* 1987) or 25 s of cycling (Sant'Ana Pereira *et al.* 1996; Karatzaferi *et al.* 2001), ATP was lower and IMP was higher in type II than in type I fibres.

In the present study using maximal exercise of shorter duration (10 s) we saw no detectable IMP production in type I fibres but systematic increases in [IMP] of IIA, IIAx and IIXa fibre populations. It is, however, notable that IMP was present in all type II fibres analysed, in which [PCr] was always over 20 mmol (kg DM)$^{-1}$, while there was an inverse linear relation between [IMP] and [PCr] (Fig. 2). This observation is in contrast to the suggestion that IMP production only occurs once PCr is totally depleted. However, this suggestion was based on data from amphibian fast muscle fibres studied *in vitro* (Nagesser *et al.* 1992) and maybe those muscle fibres were not stressed to the degree seen in the present study. The degree of reduction in PCr is indicative for the ATP turnover rate, the greater the reduction in PCr the higher the turnover rate. The production of IMP indicated that the ATP utilisation rate in this type of short-term all-out sprinting exercise was higher than the ATP resynthesis rate by the creatine kinase system and anaerobic glycolysis. Notwithstanding species and experimental differences the degree to which compartmentalisation occurs in the fibre (e.g. Seraydarian *et al.* 1962; Goudemant *et al.* 1997) during maximal exercise may give rise to localised depletion of PCr.

The experimental approach applied in this study involved the separation of single fibre fragments from needle biopsies of human muscle, the fibre type characterisation being conducted on a part of each fibre fragment, with the remainder being used for subsequent analysis of ATP, IMP, PCr and Cr content. It is a time-consuming and technically demanding process. Nevertheless the approach is critically important in seeking to understand the interaction of different muscle fibre type populations during human whole-body exercise (for example see Nevill & Greenhaff, 1999).

In the 10 s exercise used in this study there was a 23% loss of power output from the whole muscle. This was associated with almost maximal possible depletion of ATP in the IIAx and IIXa fibre populations. We believe that this suggests that these fibres may cease to contribute to mechanical power output within the first 10 s of maximal exercise. Furthermore it seems probable that the progressive whole-muscle fatigue seen in this type of maximal dynamic exercise is the consequence of sequential failure of fibre type populations in relation to their contractile and metabolic properties. It should perhaps be emphasised, however, that fibre type populations should be seen as part of a relatively 'seamless' continuum of properties rather than the discrete groups identified for the purposes of analysis (Kernell *et al.* 1983).

In conclusion, in maximal dynamic exercise of the type studied here, there is a sequential metabolic challenge first of IIX-expressing fibres, then of IIA fibres, and then of type I fibres, an observation which might be seen as supporting earlier speculation with respect to the selective fatigue of fast fibre populations following prior exercise (Beelen & Sargeant, 1991).

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